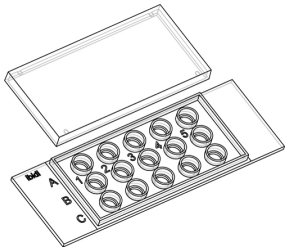


The ibidi product family is comprised of a variety of µ-Slides and µ-Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells.

The glass bottom versions of the µ-Slides and µ-Dishes are especially designed for TIRF, super resolution and single molecule applications.
The µ-Slide Angiogenesis is a cell culture product for tube formation, angiogenesis assays and direct cell culture. Cells can be grown on or in gel matrices, e.g. Matrigel™ or directly on the glass coverslip bottom.

Material

The µ-Slide Angiogenesis Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom. The µ-Slide Angiogenesis Glass Bottom is not autoclavable since it is temperature stable only up to 80°C/175°F.

Optical Properties ibidi Glass Bottom	
Refractive index n_D	1.523
Abbe number	55
Thickness	No. 1.5H (selected quality 170 µm, ± 5 µm)
Material	Schott borosilicate glass, D 263M

Shipping and Storage

The µ-Slides, µ-Dishes and µ-Plates are sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

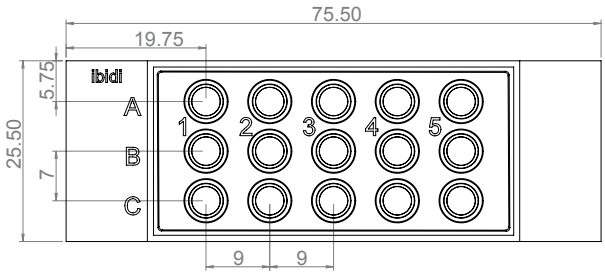
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25°C)

Shelf Life	
Glass Bottom	36 months

Geometry

The µ-Slide Angiogenesis Glass Bottom provides standard slide format according to ISO 8037/1. The well-to-well distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

Geometry of the µ-Slide Angiogenesis Glass Bottom	
Outer dimensions (w x l)	25.5 x 75.5 mm^2
Number of wells	15
Volume inner well	10 µl
Diameter inner well	4 mm
Depth inner well	0.8 mm
Volume upper well	50 µl
Diameter upper well	5 mm
Height with/without lid	5.3/3.7 mm
Growth area inner well	0.125 cm^2
Coating area using 10 µl	0.23 cm^2
Bottom	Glass Bottom



Attention!

Be cautious when handling ibidi labware products with glass bottom! The glass coverslip or glass slide is very fragile and might break easily. Handle with care to avoid physical injury and damage to devices through leakage of the medium.

Surface

The µ-Slide Angiogenesis Glass Bottom is manufactured with an uncoated glass coverslip. Washing steps (e.g. with PBS) before cell seeding can remove glass dust which is advantageous for direct cell growth on the surface.

Coating

In tube formation assays the µ-Slide Angiogenesis Glass Bottom is coated with a 0.8 mm thick layer of gel matrix.

1. Prepare your gel matrix according to the manufacturer's protocol or reference.
2. Fill the inner well with 10 µl liquid gel. Avoid air bubbles.
3. Let the gel polymerize under appropriate conditions.
4. Use as soon as possible.
5. If storage is needed fill sterile water around the wells to generate a humidified environment to hinder evaporation.

Non-gel based coatings are also possible. Please use 10 µl coating solution and calculate with an area to be coated of 0.23 cm^2 per well. Further information about coatings is provided in Application Note 08 "Cell culture coating".

Tip:

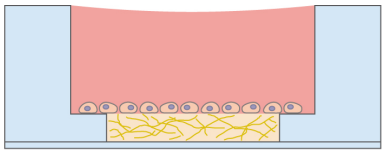
Air bubbles in the gel can be reduced by equilibrating the µ-Slide Angiogenesis Glass Bottom before usage inside the incubator overnight.
In case bent gel surfaces are created, increase or decrease the amount of gel used, until you get flat and even gels.

Tip:

For less evaporation the space in-between the wells can be filled with sterile water or agarose. Add agarose to water or buffer solution (e.g. 0.1 g to 10 ml water). Melt agarose solution using a microwave or boiling water bath and allow the solution to cool to ~50°C.

Tube Formation Assays

In a tube formation assay cells are seeded on top of the polymerized gel matrix:



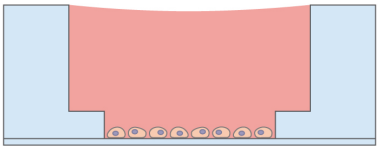
1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, we recommend 1–3 $\times 10^5$ cells/ml.
2. Apply 50 µl of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.
3. Cover the µ-Slide Angiogenesis Glass Bottom with the supplied lid. Incubate at 37°C and 5 % CO_2 as usual.
4. Conduct your experiment.
5. Depending on the cell type, medium exchange is necessary every 1–2 days. Carefully aspirate the old medium and replace it by 50 µl fresh medium.

For a detailed protocol please refer to Application Note 19 "Tube Formation" and Application Note 5 "Tube Formation in µ-Plate Angiogenesis 96 Well".

Further information about the optimization of experimental parameters and data analysis is provided in Application Note 27 "Tube Formation – Data Analysis".

Seeding Cells in 2D

You can also use the µ-Slide Angiogenesis Glass Bottom for a standard 2D cell culture without gel matrix.



1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 1.8–4.3 $\times$ 10^5 cells/ml suspension should result in a confluent layer within 2–3 days.
2. Apply 10 µl cell suspension into each well of the µ-Slide Angiogenesis Glass Bottom. Avoid shaking as this will result in inhomogeneous distribution of the cells.
3. Cover the slide with the supplied lid. Incubate at 37°C and 5 % CO_2 as usual.
4. After cell attachment, add 50 µl cell-free medium to fill the upper well.

Attention!

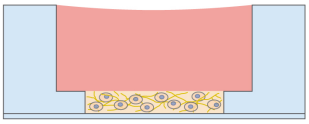
Avoid evaporation during seeding and cell culture in the incubator! We recommend placing the µ-Slide Angiogenesis Glass Bottom in an extra humidity chamber (e.g. a Petri Dish with wetted paper).

Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results might be achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace it by 60 µl fresh medium per well.

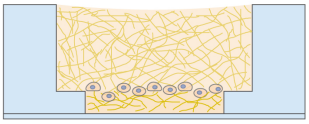
Experimental Setups

Alternatively, the µ-Slide Angiogenesis Glass Bottom can be used for the following assays:

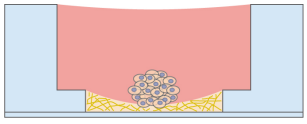
- Fill the inner well with cells suspended inside a gel matrix. After gel polymerization, add 50 µl cell-free medium to fill the upper well.



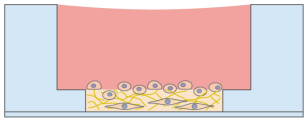
- Sandwich Cell Culture: Fill the inner well with a gel matrix. Seed cells on top of the polymerized gel and imbed the cells with 50 µl gel in the upper well.



- Fill the inner well with a low volume of the gel matrix, e.g. 8 µl. Seed cells, spheroids or tissue pieces on top of the polymerized gel. If necessary gently shake the slide to make the cells slide into the center of the well.



- Fill the inner well with fibroblasts suspended inside a gel matrix. Seed cells on top of the polymerized gel. Overlay the cell layer with medium and incubate for invasion of the cells into the gel matrix.



Microscopy

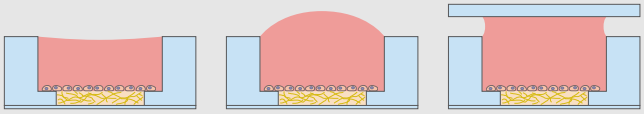
To analyze your cells, no special preparations are necessary. Cells can be directly observed live or fixed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and storage of fixed and stained samples, ibidi provides a mounting medium (50001) optimized for µ-Dishes, µ-Slides, and µ-Plates.

Note:

When gel matrices are used the optical quality and the use of high magnification objective lenses might be restricted.

Tip:

For phase contrast imaging after the experiment, the upper well can be overfilled with additional 25 µl. Closing the lid eliminates the meniscus of the upper well. This will create perfect phase contrast images. Please keep in mind that this overfilling technique might lead to well-to-well crosstalk. Therefore, we recommend this for final examination using phase contrast microscopy only.



Immersion Oil

When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

Chemical Compatibility

The table below provides some basic information on the chemical and solvent compatibility of the µ-Slide Angiogenesis Glass Bottom. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	no
Mineral oil	yes
Silicone oil	yes
Immersion oil	See **Immersion Oil** on page 4.

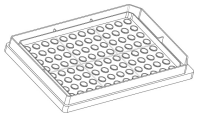
Ordering Information

The µ-Slide Angiogenesis Glass Bottom family is available with different surfaces and formats. See table below for choosing your µ-Slide and µ-Plate Angiogenesis, respectively.

µ-Slide Angiogenesis

Cat. No.	Description
81506	**µ-Slide Angiogenesis ibiTreat:** #1.5 polymer coverslip, tissue culture treated, sterilized
81501	**µ-Slide Angiogenesis Uncoated:** #1.5 polymer coverslip, hydrophobic, sterilized
81507	**µ-Slide Angiogenesis Glass Bottom:** #1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

µ-Plate Angiogenesis 96 Well



Cat. No.	Description
89646	**µ-Plate Angiogenesis 96 Well ibiTreat:** #1.5 polymer coverslip, tissue culture treated, sterilized

For research use only!

Further information can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0.